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## ORIGINAL COMMUNICATIONS.

*On the Permanent Preservation of Histological Preparations as Practised at the Army Medical Museum, Washington, D. C.* By Brevet Lt.-Col. J. J. WOODWARD, Asst. Surgeon U. S. Army. (Contributed by permission of the Surgeon General.)

All microscopists who have busied themselves with the minute anatomy of the soft tissues of the higher animals must have felt the need of an easy and reliable process by which satisfactory preparations once obtained may be permanently preserved. The various aqueous solutions, such, for example, as those proposed by Goadby, have been thoroughly tried, but have failed to preserve the tissues permanently, and thousands of preparations laboriously put up in this country and Great Britain have perished, most of them proving serviceable for but a very few months. On the other hand, alcoholic solutions of various strength not merely render the tissue opaque, but since they attack almost any cement, are soon lost by evaporation, or the specimens are preserved only at the expense of continual repairs. Much greater advantages are possessed by glycerin, which has recently been extensively used, and in which preparations keep pretty well for several months, but sooner or later, often unfortunately very soon, a peculiar granular change begins in soft tissues thus preserved, which obscures minute details, and ultimately renders the specimens useless. It is true that occasionally among these perishing glycerin preparations one holds out for a longer period; but this, if possible, increases the mortification experienced, by leading the microscopist continually to indulge in hopes which are so seldom realized.

These considerations lead the writer to believe that a few remarks on the methods of preservation found most available at the Army Medical Museum will be acceptable to microscopists.

By far the most satisfactory method of preserving specimens of most of the soft tissues is to mount them in Canada balsam, which can be effected in the majority of instances without materially changing their appearance. Where this cannot be done, however, the mixture of gum and glycerin proposed by Mr. R. J. Farrants, offers an excellent substitute; the great majority of preparations thus put up remaining unaltered for years. A few remarks may be offered on each of these methods.

1. *Farrant's gum and glycerin.*—The original formula for this mixture was as follows:[1]—

> "Boil together in a Florence flask or porcelain capsule three grains of arsenious acid and two fluidounces of distilled water: when cold filter through paper. Take of this arsenical solution one ounce, of pure glycerin one ounce, of pure gum acacia one ounce. The gum should be dissolved without heat; a fortnight or longer will be required for complete solution; in the mean time the mix-

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[1] Quarterly Journal of Microscopical Science, vol. vi., 1858, p. 119.

ture should be occasionally stirred with a glass rod. It will be well not to shake the bottle so as to froth the mixture, for air introduced is retained with great tenacity, and many days elapse before it entirely disappears. If due care be taken in selecting pieces of gum, transparent, bright, and free from impurities, the mixture will not need filtering; if, however, foreign matters have accidentally gained admission, the best substance through which to strain the mixture is fine cambric, through which a considerable quantity of clean water has been made to flow, so as to wash away any dust or loose fibres of the fabric which might find their way into the mixture."

In this mixture a large number of delicate anatomical preparations, especially perpendicular sections of ulcerated intestines, were mounted at the Army Medical Museum, between 1862 and 1865. All these preparations remain at the present time unaltered except that the colour of the red staining of those stained by imbibition with red aniline, has greatly faded and in some instances altogether disappeared, a circumstance which is the more to be regretted because of the original beauty of the preparations, and because of the facility with which the staining fluid is used. On the other hand, those stained with yellow aniline and with carmine have not changed nor have those injected with carmine. This successful preservation is in striking contrast with the fate of those specimens mounted at the Museum in pure Price's glycerin during the same period. All these have become more or less granular, and most of them have quite spoiled.

The preparations above referred to were finished with a circle of black varnish which was the more necessary as the proportion of glycerin in Farrant's formula is too great to permit the mixture to harden thoroughly. For this reason preference has been given lately to the modification of Farrant's original formula contained in Beale's *How to Work with the Microscope,*[1] which is as follows:—

Picked gum arabic and distilled water each four ounces by weight, glycerin two ounces by weight. These ingredients are to be mixed as in the original formula of Farrant, and pieces of camphor are to be laid in the stoppered bottle in which the mixture is kept. This form of gum and glycerin hardens so thoroughly in a few days that the varnish may be dispensed with, by which much time is saved when many preparations are to be put up. Dr. E. Curtis has modified the method of preparation somewhat by using a larger quantity of distilled water to dissolve the gum, and subsequently restoring the mixture to the required bulk by careful evaporation over a water-bath. The advantage of this method is that it materially shortens the time of preparation.

In mounting the soft tissues in this mixture the preparation once satisfactorily obtained in glycerin, by Beale's method, or by any other selected mode, is simply transferred by the aid of a needle and forceps to a drop of the gum and glycerin on a glass slide, and a suitable thin cover gently pressed upon it. The mixture hardens in a few days, and no varnish is necessary.

2. *Canada balsam for the preservation of the soft tissues.*—It is not necessary to say anything here in praise of Canada balsam as a means of preserving those objects whose structure remains satisfactorily visible after immersion in it. Such preparations are well known to remain unchanged for an indefinite length of time. The earlier histologists, however, were not able to mount the soft tissues satisfactorily in balsam because this substance will not mix with water, and the soft tissues when robbed of their

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[1] Fourth edition, p. 58.

moisture by drying are usually much changed, often so much so as not to be recognizable.

When at a later period, balsam was employed for the preservation of transparent injections, the charge was justly brought against it that as then used nothing but the course of the vessels was shown, and that none of the minuter details of structure were to be seen.

If, however, the water of the tissues be gradually replaced by alcohol, these changes do not occur, and the opacity produced by the alcohol is wholly removed when the specimen is subsequently transferred to turpentine preparatory to its immersion in Canada balsam. The method found most generally useful at the Army Medical Museum closely approximates that recommended by Thiersch in his work on epithelial cancer,[1] differing from it, however, in two particulars to which I am disposed to attach importance.

Thiersch hardened the morbid growth by immersing it as soon as possible after the operation or post mortem, in 85 per cent. alcohol, which was renewed after twenty-four hours, and replaced after twenty-four hours more by absolute alcohol. When the specimen became sufficiently hard he cut thin sections, stained these with carmine, by a method which will be presently detailed, transferred the stained sections again to alcohol, thence to turpentine, and finally to Canada balsam.

At the Museum it has been found expedient, as a general rule, to introduce the preparation to be hardened into weaker alcohol than recommended by Thiersch, replacing it by stronger alcohol from time to time until finally absolute alcohol is used; and it has been found desirable to use balsam, which has first been thoroughly hardened, and then dissolved in chloroform, so as to form a solution of the consistency of cream. By this method the use of heat in the balsam mounting is rendered unnecessary, and all difficulty in this part of the process is avoided.

The detailed procedure found most generally available at the Museum is as follows:—

The specimen intended for examination, for example, a portion of a morbid growth, or of some normal organ, such as the kidney or the liver, is laid in proof spirit, which is to be changed next day, and allowed to remain till it is thoroughly saturated, which seldom requires more than a few days. The bloodvessels should be previously injected with Prussian blue, or some suitable material, though many interesting details can be made out in cases where this is impossible. After lying a few days in proof spirit, the specimen is to be transferred to alcohol of 75 to 80 per cent. and thence after a few days to absolute alcohol, in which it should remain till hard enough to cut. The time required varies in accordance with the softness of the specimens from a few hours to a week or more.

After thorough hardening the piece may be kept in absolute alcohol for an indefinite period, especially if the alcohol be changed from time to time. When sufficiently hardened, sections should be cut by a thin knife, with or without the aid of any of the ordinary cutting machines. The sections thus cut are to be stained with carmine by immersing them for a short time in Thiersch's staining fluid, which is prepared as follows: Mix one part, by weight, of carmine with one of strong aqua ammonia, and three of distilled water, for the first solution; the second solution consists of one part of crystallized oxalic acid and twenty-two parts of distilled water.

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[1] Der Epithelial-Krebs. Carl Thiersch, Leipzig, 1865, p. 87.

One part of the first solution is to be added to eight of the second, and twelve of absolute alcohol, and then filtered. If the filtrate is orange, instead of carmine-red, aqua ammonia is to be added, drop by drop, until the proper colour appears. If crystals of oxalate of ammonia should form, they are to be filtered out. The solution is then ready for use.

The precise time of immersion required to stain sections successfully varies from half a minute to several minutes, and must be determined in each case by trial. When the sections have been immersed long enough they are to be washed with alcohol of 80 per cent. and then soaked in a saturated solution of oxalic acid in 80 per cent. alcohol, until the carmine remains only in the nuclei of the tissues. The time required for this varies from a few minutes to half an hour or more.

Trial having determined that the sections are soaked long enough in this fluid, those which are satisfactorily coloured are to be washed in alcohol at 80 per cent. till they are freed from all traces of oxalic acid, and then to be transferred to absolute alcohol. They are to remain in this till they have parted with all the moisture obtained during the staining process, which requires from half an hour to an hour. They are then transferred to turpentine, in which they are permitted to float till saturated, and should be immediately mounted in Canada balsam, without the use of heat. To effect this a solution of balsam in chloroform is prepared in the following manner: Evaporate some balsam over a water bath till it becomes quite solid when cold, then dissolve it in enough chloroform to give the solution about the consistency of cream. Each section is immersed in a drop of this fluid on a glass slide, and covered with a thin glass cover. The chloroform speedily evaporating leaves the balsam quite firm. The solution of balsam should be made thin enough to avoid trouble with air bubbles.

Thiersch's staining fluid, prepared as above described, has been found at the Museum to give more certain and uniform results than the simple solution of carmine in ammonia, and distilled water or glycerin, and will be preferred after trial by all to whom economy of time is an object.

Sections of morbid growths received from a distance in alcohol can be satisfactorily prepared by this method.

With regard to transparent injecting fluids it may here be remarked that the various Prussian blue fluids, including those of Beale and Thiersch, have all been found at the Museum to fade sooner or later. Experiments are now being made with a fluid suggested by Dr. J. C. W. Kennon, the basis of which is the purple of Cassius, which is a double salt of gold and tin, and which, being indestructible in acids or alkalies, quite insoluble, and of extremely fine granular constitution, will, it is hoped, be more permanent and satisfactory. The results of experiments made with this injecting fluid will be communicated hereafter.

It may be mentioned in conclusion that specimens stained with nitrate of silver after the plan of Recklinghausen, or with chloride of gold as recommended by Cohnheim, can be readily transferred to Canada balsam, and so permanently preserved. The same is true of glycerin preparations made after the method of Dr. L. Beale. The principle to be observed in any case is to transfer the specimen to weak alcohol, which is to be replaced from time to time by stronger, until by the ultimate action of absolute alcohol all the water is removed. It is then to be transferred to turpentine, which speedily replaces the alcohol, and thence to Canada balsam. The object of the gradual transfer from weaker to stronger alcohol, is to rob the tissue of its water by degrees, a precaution by which the shrinking and collapse, consequent upon sudden immersion in absolute alcohol, is completely avoided. The time required for this purpose varies with the size of the preparation. In the case of thin sections or fragments of tissues, suitable for the higher powers of the microscope, a few minutes in each fluid is often sufficient.

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### *Case of Placenta Prævia, successfully treated by Simpson's Method.* By John W. Booth, M. D., of Tally-Ho, North Carolina.

On the 22d of June, 1868, I was hastily summoned, about sunrise, to visit, with Dr. Cozart, the family physician, Mrs. R., about six months advanced in her seventh pregnancy, who had been suddenly attacked, the previous evening, with profuse and alarming uterine hemorrhage, which had almost ceased spontaneously before the arrival of Dr. C. There had been a very slight discharge of blood during the night and until I saw her. We both remained with the patient until the morning of the 23d, when leaving her to the care of Dr. C., I made some necessary calls, and returned late in the afternoon to take charge of her during the night, that Dr. C., might attend to his most urgent duties, and get back, we *hoped*, before the time of greatest need. During the whole of this time after my arrival, although very little had been done in the way of treatment, there had been no discharge and only occasional very slight uterine contraction. From the symptoms, we strongly suspected that the flooding proceeded from placenta prævia. The uterus was too high up to be reached by a common digital examination, and the urgency of the case did not yet require any further. Hence we deemed it unsafe to leave the patient without professional assistance at hand.

About 10 o'clock, having retired to bed in an adjoining room, I was awakened and informed that my presence was required, as Mrs. R. was flooding prodigiously. I lost no time, and found her almost in extremis. The bed was deluged with blood. The lady's cheeks and prolabia were blanched; she was nearly pulseless, &c.; in short, almost in a state of collapse from loss of blood, and that after a few pains, which she described as only a "drawing sensation."

I now introduced my hand into the vagina sufficiently to pass my two fingers through to the placenta, which was implanted centrally over the internal os. With my finger I detached the placenta and withdrew it, not without some difficulty. The internal os, which was barely dilated enough to admit two fingers, contracted from the irritation and impeded the movements of the finger. The flooding immediately ceased, and the patient was soon delivered of a small dead fœtus. She did very well, never having a bad symptom after the detachment of the placenta.

November 6, 1868.

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### *Gunshot Wound of Bladder and Rectum; Recovery of Patient under Remarkable Circumstances.* By John D. Jackson, M. D., of Danville, Kentucky.

The following case, which we report from notes taken at the time of application for discharge by the subject of it, we chronicle as another and very remarkable instance to be added to the catalogue of cases of the wonderfully recuperative power sometimes exhibited by our species.

Michael Tipps, æt. 21, private of Co. A., 17th Tennessee Regiment, Confederate States Army, on the 26th of November, 1862, applied for a discharge from military service, because of physical disability, resulting